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Effects of Soil Moisture on Ecophysiological Characteristics of *Adiantum reniforme* var. *sinensis*, an Endangered Fern Endemic to the Three Gorges Region in China

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ABSTRACT.—The effects of soil moisture (80%, 60% and 40% water holding capacity) on dry matter production and allocation, leaf morphological and physiological characteristics were examined in *Adiantum reniforme* var. *sinensis*, an endangered fern endemic to the Three Gorges region in southwest China. Drought stress decreased leaf growth and photosynthetic capacity, and hence reduced total mass, specific leaf area (SLA) and leaf area ratio (LAR). Dry matter allocation into the root fraction, however, increased with decreasing soil moisture. Leaf relative water content (RWC) decreased as soil water depletion, but the differences were insignificant. Such results might be the result of a physiological balance between the demand for water by the leaves and the water uptake from soil by the roots. The decrease in stomatal conductance (g_s) effectively controlled water loss and maintained intrinsic water use efficiency (WUE_i) under drought stress. The increase in proline content might contribute to osmotic adjustment, and hence sustained cytomembrane integrality in structure and function under drought conditions.

KEY WORDS.—*Adiantum reniforme* var. *sinensis*, ecophysiological characteristics, fern, soil moisture

Depending on environmental conditions, plants can alter their morphology, biomass allocation, and physiological processes to adapt to changing environments (Via *et al.*, 1995; Sultan, 2001). In order to evade or decrease the influences of water stress, plants can alter various functional traits such as: root/shoot ratio (R/S), specific leaf area (SLA) (Turner, 1997; Marcelis *et al.*, 1998; Liu and Stützel, 2004), stomatal regulation, and osmotic adjustment (Liu and Stützel, 2002a; 2002b). However, there are few studies that address the ability of ferns to respond to soil water deficiency.

Adiantum reniforme var. *sinensis* Y.X. Lin, an evergreen fern of the family Adiantaceae, is only distributed at elevations 80–480 m in Wanzhou District and Shizhu County of Chongqing Municipality (Xie, 1993; Shi *et al.*, 2005). The range of this species lies within the Three Gorges region of Yangtze River, China. Due to overexploitation and the Three Gorges Project construction, the number and distribution of *A. reniforme* var. *sinensis* are decreasing quickly and this species is now listed as endangered in the Chinese red data book (Fu, 1992).

The main habitats of *A. reniforme* var. *sinensis* are cliffs or steeply sloped rocks with thin soil. Such conditions result in frequent water stress (mean soil

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water content is 12.8 ± 2.2% (w/w), approximately 50% of water holding capacity). Previous studies of *A. reniforme* var. *sinensis* have focused on spore propagation and genetic diversity (Xie, 1993; Xu *et al.*, 1998; Pan *et al.*, 2005). Little work has been done on the ecophysiological aspects of the species, especially as it relates to its ability to grow in such drought prone habitats.

The objectives of this study were to investigate the effects of soil moisture on dry matter production and allocation, leaf morphological and physiological characteristics. We hypothesized that drought stress could change these ecophysiological characteristics differently, and these changes might compensate for the shortage of water during drought periods. The information obtained will be useful in designing an effective conservation plan for *A. reniforme* var. *sinensis*.

Material and Methods

Plant material and growth conditions.—Unlike other members of the genus, *A. reniforme* var. *sinensis* is a single-leaf fern, 5–20 cm tall. The leaves are circular or kidney-shaped (2–6 cm in diameter). Spores of *A. reniforme* var. *sinensis* were collected from Wanzhou District (30°30′N, 108°17′E), Chongqing, Southwest China, and germinated in October 2004 in the laboratory. When sporelings reached 2.5 cm (17 months after germinating), the healthy and uniform plants were selected and transplanted into 21 cm (diameter) × 15 cm (height) pots (six sporelings per pot), filled with a mixture of leaf mold, peat soil and sand (2:1:1, v/v/v). The pots were placed in a growth chamber with a 13 h photoperiod, a photon flux density (PFD) of 360 μmol m^{-2} s^{-1}, 70% relative humidity and 24°C air temperature. These values represented the mean values of natural habitats of *A. reniforme* var. *sinensis*. Three soil water treatments were started on March 25, 2006 and each treatment had three replicates. For the well-watered treatment, plants were watered to 20.6% (80% of water holding capacity (WHC)). Similarly, for the middle and severe water-stress treatments, water was added to 15.5% (60% of WHC), 10.3% (40% of WHC), respectively. The pots were watered every other day and the soil water contents were controlled by commonly-used weight method.

Measurements.—Six weeks later (10 May 2006), Photosynthetic PFD-response curves were measured with a portable gas exchange measuring system (LI-6400, Li-Cor, Lincoln, USA). CO_2 and air temperature in the leaf chamber were maintained at 360 μmol mol^{-1} and 24°C, respectively. PFD started at 800 μmol m^{-2} s^{-1} and decreased stepwise to 0 μmol m^{-2} s^{-1}. A time interval of 90 s was given for the leaf to equilibrate to the new conditions in each measurement. Saturation PFD, compensation PFD, and PFD-saturated photosynthetic rate (P_{max}) were estimated. Leaf intrinsic water use efficiency (WUE_i) was calculated using P_{max} and stomatal conductance (g_s) at saturation PFD. Chlorophyll (Chl) was extracted using ethanol and acetone (1:2, v/v). The concentrations of Chl *a* and Chl *b* in extracts were determined from absorbances at 663 and 645 nm, respectively, with a UV-2100 spectrophotometer (Unico, Shanghai, China). Leaf relative water content (RWC) was

TABLE 1. Effects of soil moisture on dry matter accumulation and total leaf area (LA) of *A. reniforme* var. *sinensis*. Data are the means ± SE of six replicates. Different letters in each column indicate significant differences ($P < 0.05$).

Treatments (%WHC)	Dry matter accumulation (g)				LA (cm^2 $plant^{-1}$)
	Root	Stem	Leaf	Total	
80%	0.06 ± 0.02^a	0.06 ± 0.02^a	0.20 ± 0.02^a	0.33 ± 0.01^a	54.29 ± 9.30^a
60%	0.08 ± 0.02^a	0.05 ± 0.00^a	0.17 ± 0.03^{ab}	0.30 ± 0.05^{ab}	36.56 ± 9.57^b
40%	0.08 ± 0.01^a	0.05 ± 0.01^a	0.14 ± 0.02^b	0.27 ± 0.04^b	26.98 ± 4.75^c
P-Value	0.273	0.077	0.012	0.049	0.001

measured as $[(W_f\text{-}W_d)/(W_s\text{-}W_d) \times 100]$ according to Barrs and Weatherley (1962). Membrane stability index (MSI) was calculated as $(1-C_1/C_2) \times 100$ according to the method of Sairam *et al.* (1997/1998). Five leaf discs (0.7 cm diameter) were immersed in twice-distilled water at 24°C for 30 min. The electrical conductivity (C_1) was recorded by a DDS-11A conductormeter (Shanghai, China). After 15 min boiling water bath, the conductivity was measured when the temperature reduced to about 24°C (C_2). Proline content in leaves was extracted in 3% aqueous solution of sulphosalicylic acid and determined by the method of Zhang *et al.* (1990). After all measurements were made, six intact individuals were harvested from the three replicate pots. Total leaf area (LA) was determined using a portable leaf area meter (Li-Cor-3000, Lincoln, NE, USA) before all samples were dried in an oven at 80°C for at least 72 h. Leaf area per unit leaf mass (specific leaf area, SLA), leaf area per unit of total mass (leaf area ratio, LAR), root mass per unit of total mass (root mass ratio, RMR) and root mass/shoot mass (R/S ratio) were determined according to Hunt (1978).

Statistical analysis.—Statistical analysis was conducted using SPSS 13.0 for windows (SPSS Inc., Chicago, USA). All means were expressed with their standard error (± SE) and compared using one-way ANOVA followed by least significant difference (LSD) post-hoc analysis ($P < 0.05$).

RESULTS

Dry matter production and allocation.—The differences in root and stem mass between the water treatments were insignificant, but leaf and total mass were significantly lower under 40% WHC than that under 80% WHC (Table 1). LA was significantly smaller under lower water treatments. The ratios of leaf area to leaf mass (SLA) and to total mass (LAR) were also smaller under lower water treatments (Fig. 1). RMR and R/S, however, increased with the decrease of soil moisture.

Photosynthetic characteristics and water use efficiency.—For three soil water treatments, net photosynthetic rate (P_n) increased rapidly and reached their constant at similar saturation PFD (Fig. 2, Table 2). P_{max}, however, decreased as soil moisture decreased and there was a significant difference between 40% and 80% WHC treatments (Table 2). Contrarily, compensation

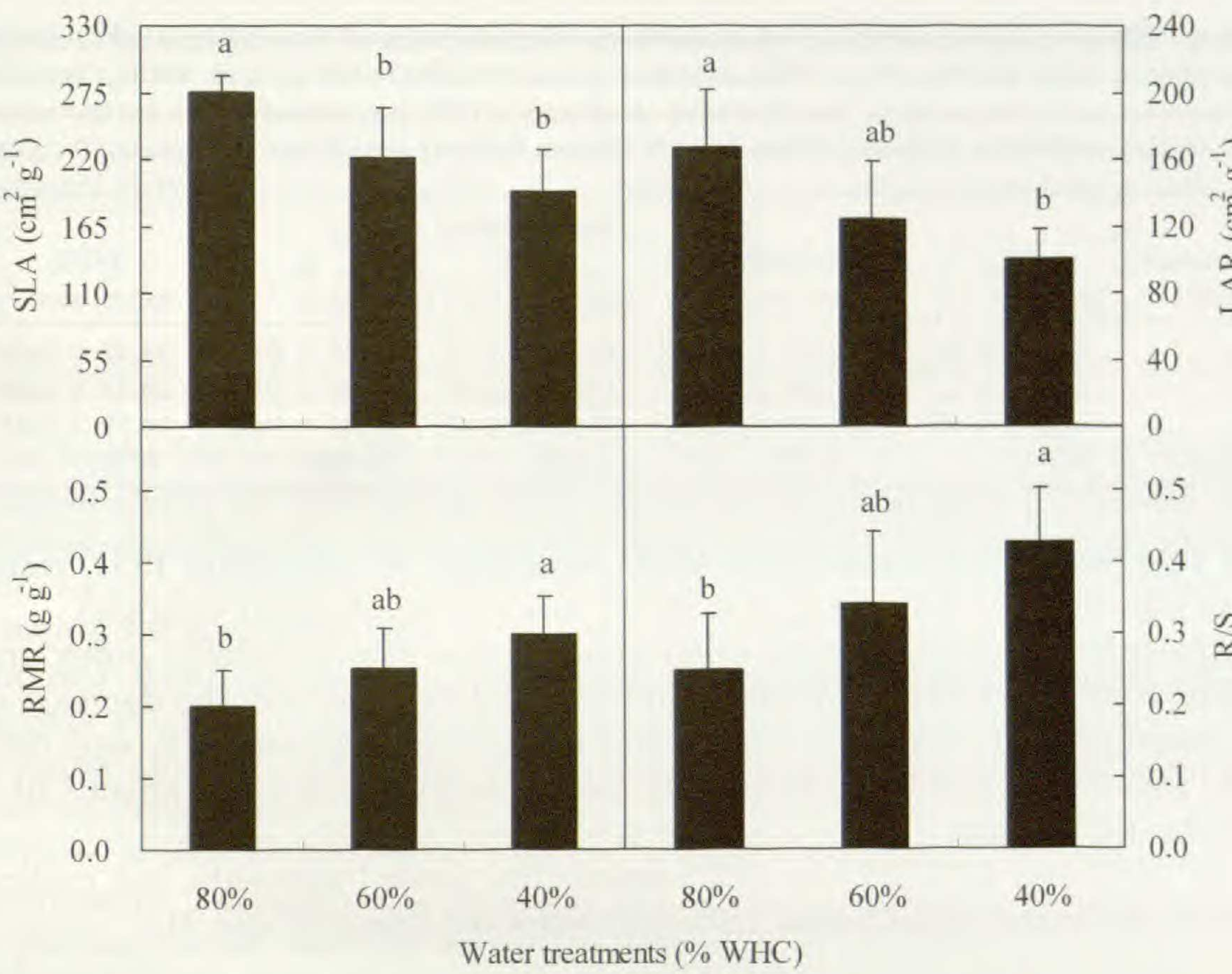

FIG. 1. Effects of soil moisture on leaf and root characteristics of *A. reniforme* var. *sinensis*. SLA, specific leaf area; LAR, leaf area ratio; RMR, root mass ratio; R/S, root/shoot ratio. Data are the means ± SE of six replicates. Different letters in each graph indicate significant differences ($P<0.05$).

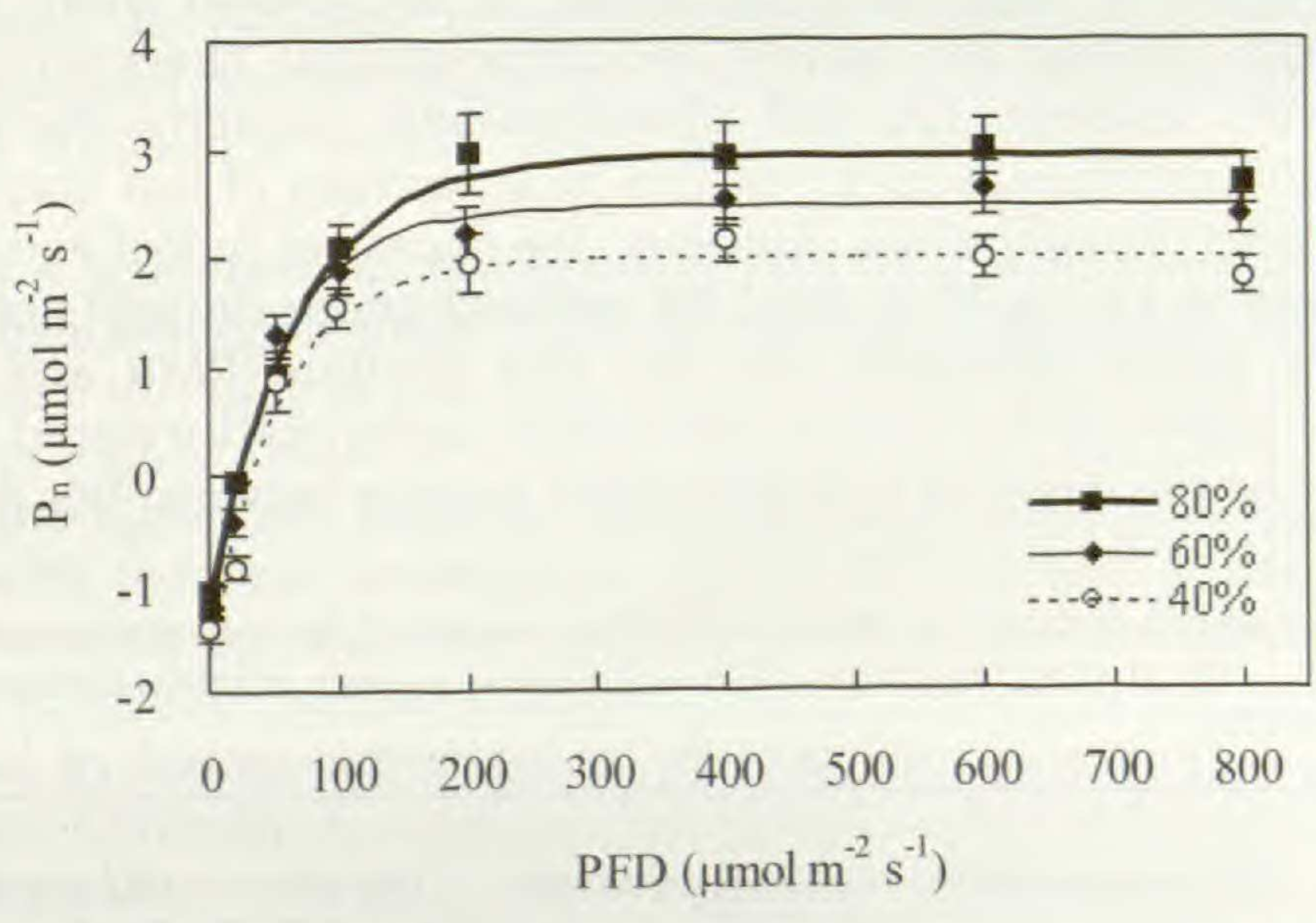

FIG. 2. Effects of soil moisture on photon flux density (PFD) response curve of net photosynthetic rate (P_n) of *A. reniforme* var. *sinensis*. P_n were measured at CO_2 concentration of 360 μmol mol^{-1} and temperature of 24 °C, with PFD values ranging from 0 to 800 μmol m^{-2} s^{-1}. Data are the means ± SE of three replicates.

TABLE 2. Effects of soil moisture on photosynthetic characteristics of *A. reniforme* var. *sinensis*. PFD, photon flux density; P_{max}, PFD-saturated photosynthetic rate; g_s and WUE_i, stomatal conductance and intrinsic water use efficiency at saturation PFD, respectively. Data are the means ± SE of three replicates. Different letters in each column indicate significant differences (P<0.05).

Treatments (%WHC)	P_{max} (μmol m^{-2} s^{-1})	Saturation PFD (μmol m^{-2} s^{-1})	Compensation PFD (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	WUE_i (μmol mol^{-1})
80%	2.90 ± 0.29^a	313.51 ± 31.66^a	21.49 ± 2.17^b	0.12 ± 0.01^a	24.82 ± 2.51^a
60%	2.44 ± 0.25^{ab}	274.56 ± 27.73^a	22.57 ± 2.28^b	0.09 ± 0.01^b	28.47 ± 2.88^a
40%	1.98 ± 0.20^b	251.43 ± 25.39^a	30.67 ± 3.10^a	0.07 ± 0.01^b	28.39 ± 2.87^a
P-Value	0.011	0.091	0.009	0.002	0.257

PFD was the highest under 40% WHC condition. At saturation PFD, water stress significantly reduced g_s, but WUE_i was not significantly changed.

Chlorophyll content, relative water content, membrane stability index and proline content.—Content of Chl *a* and Chl *a+b* decreased with the decrease of soil moisture and there were significant differences between 40% and 80% WHC treatments (Table 3). Soil water status, however, did not affect Chl *b* content and Chl *a*/*b*.

Leaf RWC and MSI did not differ among the water treatments, but proline content increased significantly with soil water depletion (Table 4).

DISCUSSION

Phenotypic plasticity plays an important role in the ability of plants to adapt to changing environments by buffering the effect of natural selection acting on genotypes (Scheiner, 1993; Ge *et al.*, 2004). In the present study, soil water status strongly affected the growth and physiological characteristics of *A. reniforme* var. *sinensis*. LA and photosynthetic capacity declined with decreasing soil moisture, which resulted in a decrease of leaf mass and thus total mass under drought stress. However, the decreases in leaf and total mass were less than in LA, so SLA and LAR reduced under drought stress too. In contrast, dry matter allocation into the root fraction (RMR and R/S) was significantly higher under drought stress than under well-watered treatment. These might be the result of a physiological balance between the demand for

TABLE 3. Effects of soil moisture on chlorophyll (Chl) content of *A. reniforme* var. *sinensis*. Data are the means ± SE of three replicates. Different letters in each column indicate significant differences (P<0.05).

Treatments (%WHC)	Chl *a* (mg g^{-1}DW)	Chl *b* (mg g^{-1}DW)	Chl *a*/*b*	Chl *a+b* (mg g^{-1}DW)
80%	3.23 ± 0.31^a	1.66 ± 0.16^a	1.94 ± 0.07^a	4.89 ± 0.46^a
60%	2.84 ± 0.11^a	1.48 ± 0.16^a	1.93 ± 0.19^a	4.32 ± 0.22^{ab}
40%	2.58 ± 0.11^b	1.36 ± 0.12^a	1.89 ± 0.07^a	3.94 ± 0.12^b
P-Value	0.022	0.080	0.894	0.024

TABLE 4. Effects of soil moisture on leaf relative water content (RWC), membrane stability index (MSI) and proline content of *A. reniforme* var. *sinensis*. Data are the means ± SE of three replicates. Different letters in each column indicate significant differences ($P<0.05$).

Treatments (%WHC)	RWC (%)	MSI (%)	Proline content ($\mu g\ g^{-1}DW$)
80%	80.91 ±10.55^a	82.85 ± 1.96^a	227.76 ± 23.00^c
60%	70.89 ± 9.19^a	80.79 ± 3.35^a	303.96 ± 30.70^b
40%	62.06 ± 8.51^a	78.71 ± 2.09^a	397.46 ± 40.14^a
P-Value	0.126	0.218	0.002

water by the leaves and the water uptake from soil by the roots. The RWC, the common index for estimating water status in leaves (Hsiao, 1973), did not decrease significantly under drought stress, further indicated maintaining water balance may be one strategy for *A. reniforme* var. *sinensis* to acclimate to changing water conditions.

In this study, g_s and P_{max} decreased significantly under drought stress, whereas WUE_i did not statistically change and the values increased. This indicated stomatal regulation of *A. reniforme* var. *sinensis* under drought stress decreased photosynthesis but effectively controlled water loss. With the increase of soil water stress, Chl *a* decreased but Chl *b* did not change significantly. Therefore, the decreased Chl *a* content might be another reason for photosynthesis decrease under drought.

Water deficit triggers the accumulation of proline in many species (Delauney and Verma, 1993; Yin *et al.*, 2005; Bertamini *et al.*, 2006). Significantly higher proline contents in lower soil moisture were also found in *A. reniforme* var. *sinensis*. The accumulation of proline may contribute to maintaining proper balance between extra-cellular and intracellular osmolarity under water stress. Therefore MSI, an indicator of cytomembrane integrality in structure and function, remained almost unaffected compared to normal water treatment, could partly attribute to osmotic adjustment such as proline.

In conclusion, dry matter allocation, leaf morphological and physiological responses of *A. reniforme* var. *sinensis* were beneficial to acquire and utilize soil water and hence survive under critically drought conditions. However, the fitness-related traits, such as total mass, photosynthetic capacity and chlorophyll content, were highest under 80% WHC, which revealed that *A. reniforme* var. *sinensis* favors higher soil moisture for its photosynthetic carbon gain. This is consistent with previous investigation, which showed that *A. reniforme* var. *sinensis* grew better in moist but well-drained slope habitats than others (Xie, 1993; Shi *et al.*, 2005). The favored habitats, however, are few and dispersed and are disturbed by human activities and the ongoing construction of the Three Gorges Project. Currently, *A. reniforme* var. *sinensis* is mainly distributed on cliffs or steeply sloped bare rocks with thin soil, which water stress appears frequently and few human disturbances (Pan *et al.*, 2005; Shi *et al.*, 2005). Thus, for the effective conservation of the endangered species, a management of the remaining natural habitats or *ex-situ* conservation should be taken to provide sufficient water availability which is essential for efficient biomass accumulation.

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